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A General, Highly Efficient Azeotropic Method of Esterification of Amino Acids

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Received September 2, 1970

For the large-scale preparation of amino acid esters the classical method using anhydrous alcohol and an acid catalyst is expensive, time consuming, and prone to yields which are far from quantitative. Further more, the yields are quite variable from batch to batch. Since the reaction system involves an equilibrium in which the presence of water will reverse the reaction, the rapid removal of water from the reaction system should improve both the rate of completion of the esterification and the yield. Therefore, an azeotropic distillation technique involving the alcohol-benzene-water azeotrope was used to promote the removal of the water.

METHODS

A 500 ml round-bottomed two-necked flask is equipped with a magnetic stirring bar, thermometer, and condenser with water separator and calcium chloride tube. The flask is then set into a silicon or oil bath and placed on a magnetic stirrer-hot plate. Into the flask are placed 0.1 mole of an amino acid, or its hydrochloride, 200 ml 95% ethyl alcohol, 150 ml benzene and 20–30 ml concentrated hydrochloric acid. The amount of acid should be sufficient to neutralize the amino group and create a 1.5–2.0 *N* solution, since the velocity of esterification depends upon the concentration of the catalyst. The oil bath is then heated at 80–85°C, and the esterification is allowed to progress under constant stirring and simultaneous distillation of the azeotropic mixture, which begins to distill at 66°C. In about 30 min, 200 ml of the azeotropic mixture is separated, and the temperature rises to about 72°. At that point, 50 ml alcohol and 150 ml benzene (1) are added and the distillation is continued for another 30 min, until the temperature rises to about 72–74°, and an additional 200 ml of the azeotropic mixture is collected. The reaction mixture is maintained at that temperature, under slow reflux, for 90 min, then 50 ml each of alcohol and benzene is added, and within

15 min an additional 100 ml of the azeotropic mixture is collected. The reaction mixture is maintained at slow refluxing for an additional 60 min; then the temperature of the bath is lowered to about 50°, and the reaction mixture is concentrated at reduced pressure, under nitrogen, until a dry, or oil-like, residue is obtained. After cooling, the residue is stirred with 100 ml ether, filtered by suction, and dried at about 50° and 0.1 mm Hg. It must be mentioned that alanine and lysine hydrochlorides give usually oil-like residues, which on stirring with ether convert, easily, to solids. Arginine ethyl ester dihydrochloride yields a wax-like residue, which can be converted into a solid by expanding under a high vacuum, to a foam-like state, and allowing it to solidify for several hours in this condition. No method was found to convert proline ethyl ester hydrochloride into a solid.

The hydrochlorides can be easily converted into the free esters, with good yields, utilizing the method developed by Hillman (2).

RESULTS

Azeotropic esterification of amino acids was found to eliminate the requirements for a high grade of absolute alcohol and anhydrous hydrogen chloride gas, shorten the time of esterification, and give excellent yields of high-purity products, as is shown in Table 1.

To obtain the highest yields, it was found necessary to make the periodic additions of alcohol and benzene and permit some reflux time as outlined in the method rather than complete the removal of the water azeotrope rapidly in a single distillation.

If one starts with an amino acid of good purity, one obtains an ester

TABLE 1
Data on Ester Hydrochlorides of Selected Amino Acids

Ethyl ester hydrochlorides of	Literature		Present method[a]		
	m.p., °C	ref. no.	m.p.,[b] °C	N (found)	N (calcd.)
Alanine	64–8	15	71–3	9.05	9.11
	69–72	16			
Arginine	—	17	66–9	20.38	20.36
Glycine	144	18	141–3	9.85	10.03
Lysine	144	19	140–2	11.27	11.35
Proline	oil	20	oil	7.70	7.78
Tyrosine	166	18	167–8	5.58	5.70

[a] Data obtained on unrecrystallized products.

[b] The melting point of tyrosine ethyl ester hydrochloride was determined on a Fisher Johns melting point apparatus. The melting points of the other compounds were determined in a vacuum capillary. The melting points are not corrected.

than hydrogen chloride gas. It becomes self-evident that hydrogen chloride would be the preferred catalyst if toward the end of esterification a more anhydrous medium could be created.

The present study has established that the well-known azeotropic system, alcohol-benzene-water provides desirable anhydrous conditions, and makes possible the use of commercial concentrated hydrochloric acid as the catalyst instead of the more expensive anhydrous hydrogen chloride gas. In addition, this procedure allows the use of 95% alcohol in place of the more expensive absolute alcohol. Other azeotrope-forming alcohols can also be used.

Since slight additions of fresh alcohol and benzene are required to achieve the highest yields, it appears that too rapid attainment of a completely anhydrous system may retard the esterification and prevent attainment of high yield. It thus appears that hydronium ion is more efficient than alcoxonium ion in catalyzing the esterification.

SUMMARY

A highly efficient general method for the esterification of amino acids has been developed. Simultaneous esterification and azeotropic distillation of the alcohol-benzene-water azeotrope permits the use of 95% alcohol and ordinary concentrated hydrochloric acid. The method described provides quantitative yields of high-purity ester hydrochlorides. The results from the esterification of alanine, arginine, glycine, lysine, proline, and tyrosine are presented.

ACKNOWLEDGMENT

We wish to thank Mrs. Marta Lukasewycz for the nitrogen analyses.

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whose purity, without recrystallization, is equal to the purified material described in the literature. The nitrogen values obtained on the original products indicate that these materials possess sufficient purity, for most practical purposes, without further purification.

In general, the ethyl ester hydrochlorides of most amino acids are hygroscopic materials, especially those of arginine and lysine; however, hygroscopicity significantly decreases as purity increases, i.e., when traces of the trapped hydrogen chloride are removed. This was observed earlier by T. B. Johnson (3), on the ester of alanine.

DISCUSSION

Esters of amino acids, in particular ethyl esters, have been known for several decades (4–7), and the general procedure for their synthesis, adopted from Curtius (4) and Fischer (7), involves an extensive refluxing of an amino acid with a large excess of absolute alcohol while bubbling through the reaction mixture anhydrous hydrogen chloride gas, which catalyzes the reaction.

Toward the end of esterification the clear solution represents an equilibrated system between rate of formation and hydrolysis of the ester. At that point, most of the acid is esterified, but a considerable amount remains in a solvated form, due to the exceptional ease of hydrolysis. Also, assumptions can be made that, under these conditions, a double-charged cation is formed (8) that hinders further esterification.

Due to the process of constant hydrolysis one obtains rather low yields of the esters, and the situation does not improve much if the reaction is carried longer, even for several hours, unless the water of esterification is removed. Of course this cannot be achieved under the classical conditions of esterification. In addition, the large quantities of absolute alcohol and anhydrous hydrogen chloride gas, used in that procedure, are impractical for large-scale preparations.

Numerous attempts have been made to improve the classical condition of esterification, utilizing different catalysts (3,10), *trans*-esterifications (9,11), and strong cation-exchange resins (12). Also, attempts were made by Ishizuka and Taguchi (13) to develop a method utilizing a highly anhydrous medium, consisting of concentrated sulfuric acid and phosphorus pentoxide.

In general the basic catalysts, in particular alkoxides, possess only a limited applicability, due to the fact that these catalysts cause racemization (9,14). Other acid catalysts, such as $SOCl_2$ SO_2Cl_2, RSO_2Cl, PCl_3, and $POCl_3$ (10), slow down the process of esterification, give lower yields, and are suitable only for selective esterification of *N*-acetylated amino acids and peptides. In addition these catalysts are much more expensive